

Characterization of a cryptic *IGH/CCND1* rearrangement in a case of mantle cell lymphoma with negative *CCND1* FISH studies

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Key Points

- This article characterizes a cryptic *IGH/CCND1* rearrangement in MCL by NGS.
- Mate-pair sequencing can help in accurately diagnosing MCL in cases of cyclin-D1–positive B-cell lymphoma with negative *CCND1* FISH studies.

Introduction

Mantle cell lymphoma (MCL) is a mature B-cell neoplasm accounting for approximately 3% to 10% of all non-Hodgkin lymphomas.^{1–3} The molecular hallmark of MCL, t(11;14)(q13;q32) (*IGH/CCND1*), results in deregulation and overexpression of the G₁-phase cell cycle gene, *CCND1* (encoding cyclin D1), mediated by enhancer elements found throughout the *IGH* locus.^{1–4} The t(11;14) is observed in >95% of MCL cases, with rare reports that describe t(11;14) as being a result of *CCND1* and immunoglobulin light chain translocations.¹ Cyclin D1–negative MCL resulting from *CCND2* or *CCND3* rearrangements and immunoglobulin partners highlights the importance of cyclin D overexpression in all MCL subtypes.^{1,3–6} However, overexpression of cyclin D alone is insufficient to cause MCL, and additional genetic alterations that deregulate cell cycle, DNA damage response, apoptosis, and/or chromatin modifiers are typically required.^{1,3,7–12} Although cyclin D1 overexpression can occur in other mature B-cell lymphomas such as hairy cell leukemia and rarely chronic lymphocytic leukemia/small lymphocytic lymphoma, *IGH/CCND1* fusion detected in mature B-cell lymphomas has been considered diagnostic for MCL.^{1,13,14}

IGH/CCND1 rearrangements are typically detected by fluorescence in situ hybridization (FISH) studies using dual-color dual-fusion (D-FISH) or less commonly by *CCND1* break-apart probe sets. We describe a unique case of cyclin D1–positive B-cell lymphoma with partial features of MCL but without *CCND1* rearrangement by commercially available FISH probe sets. To further interrogate this discrepancy, a next-generation sequencing (NGS) strategy, mate-pair sequencing (MPseq), was performed, and it revealed a cryptic *IGH/CCND1* rearrangement.

Case description

A 79-year-old woman presented with fatigue, decreased appetite, and dizziness of 2-week duration. Her hematologic laboratory profile was significant for a total white blood cell (WBC) count of $94.8 \times 10^9/L$ (95% lymphocytes), hemoglobin 10.1 g/dL, and platelets $143 \times 10^9/L$. A computed tomography scan of chest, abdomen, and pelvis and positron emission tomography scan demonstrated generalized lymphadenopathy and splenomegaly. FISH studies revealed *TP53* deletion but were negative for *IGH/CCND1* fusion and *CCND2* rearrangement. *CCND3* rearrangement studies were not performed. Hematopathologic review demonstrated strong, uniform overexpression of cyclin D1, positivity for CD5 and a lack of CD200 expression by the neoplastic lymphocytes, which support a diagnosis of MCL. The lack of SOX11 expression by the lymphoma cells is highly uncommon in MCL, although it can occur in indolent or *TP53* mutation–associated MCL.^{15,16} Because cyclin D1 overexpression by lymphoma cells is not 100% specific for MCL in the absence of *IGH/CCND1* fusion, an initial diagnosis of cyclin D1–positive B-cell lymphoma was established, and the patient was subsequently treated with ibrutinib.

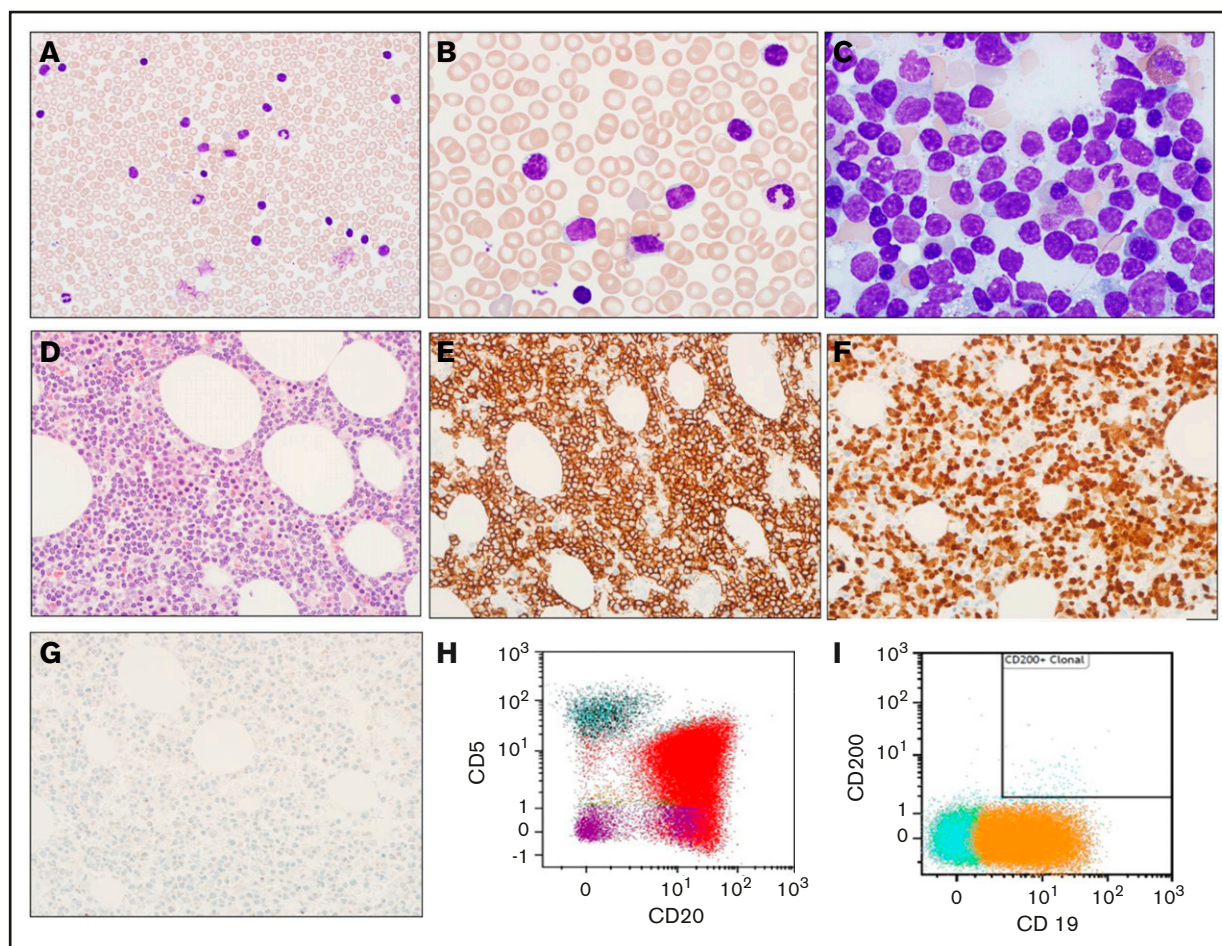


Figure 1. Morphologic and immunophenotypic evaluation. (A-B) Low (original magnification $\times 400$) and high (original magnification $\times 1000$) magnification of Wright-Giemsa stained peripheral blood smear show circulating small lymphocytes, some of which contain cytoplasmic vacuoles. (C) Wright-Giemsa stained bone marrow aspirate smear at high (original magnification $\times 1000$) magnification shows similar small lymphocytes with cytoplasmic vacuoles. (D) Hematoxylin and eosin-stained bone marrow biopsy (original magnification $\times 400$) shows extensive infiltration with small lymphocytes. (E-G) Immunohistochemical stains indicate that lymphocytes are positive for CD20 (E) and cyclin D1 (F) but are negative for SOX11 (G). (H-I) Immunophenotyping by flow cytometry demonstrates λ light chain-restricted B lymphocytes are positive for CD20 (red) and CD19 (orange), partially positive for CD5, and negative for CD200.

After 18 months of ibrutinib therapy with partial remission, the patient progressed with a rapidly rising WBC count ($119.5 \times 10^9/L$; 89% lymphocytes). No evidence of acquired *BTK* or *PLCG2* mutations were detected in the peripheral blood. The peripheral blood smear revealed marked lymphocytosis with predominantly small lymphocytes (85% of total cells) with condensed chromatin, scant cytoplasm, and prominent cytoplasmic vacuoles (Figure 1A-B). The bone marrow was hypercellular (approximately 70%) with 70% to 80% involvement by the mature lymphocytes (Figure 1C-D). The lymphocytes were CD20⁺, coexpressed cyclin D1, lacked expression for SOX11 (Figure 1E-G), and also lacked hairy cell leukemia-associated markers tartrate-resistant acid phosphatase, DBA4.4, T-bet, and annexin A1 (data not shown). Flow cytometry revealed λ light chain-restricted B lymphocytes, positive for CD20 and CD19, partially positive for CD5, and negative for CD200 (Figure 1H-I). FISH analysis revealed *TP53* and 13q deletions, gain of *BCL3*, and an extra *IGH* signal in $\sim 59\%$ to 77% of interphase nuclei (supplemental Table 1). The *IGH/CCND1* D-FISH and *CCND1* break-apart probe studies were negative (Figure 2A-B).

MPseq and a 61-gene NGS panel were subsequently performed on the bone marrow specimen.

Methods

All FISH probes were supplied by Abbott Molecular (Des Plaines, IL) with the exception of our laboratory-developed *IGH/BCL3* D-FISH probe set (details provided in supplemental Table 1). For MPseq, DNA was processed using the Illumina Nextera Mate Pair library preparation kit (Illumina, San Diego, CA) and sequenced on the Illumina HiSeq 2500 using 101 base pair reads and paired-end sequencing. Data were aligned to the reference genome (GRCh38) using BIMA V3, and abnormalities were identified and visualized using in-house-developed bioinformatics tools, SVAtools, and in-house-developed visualization tool, Ingenium.^{17,18} NGS was performed using a 61-gene panel (supplemental Table 2). Approximately 200 ng of genomic DNA sheared to approximately 150 base pairs was used for library preparation by the SureSelect XT library kit (Agilent, Santa Clara, CA) and sequenced on the HiSeq platform (Illumina) using 2×101 read lengths. Data were aligned to the reference genome (GRCh38) and processed through

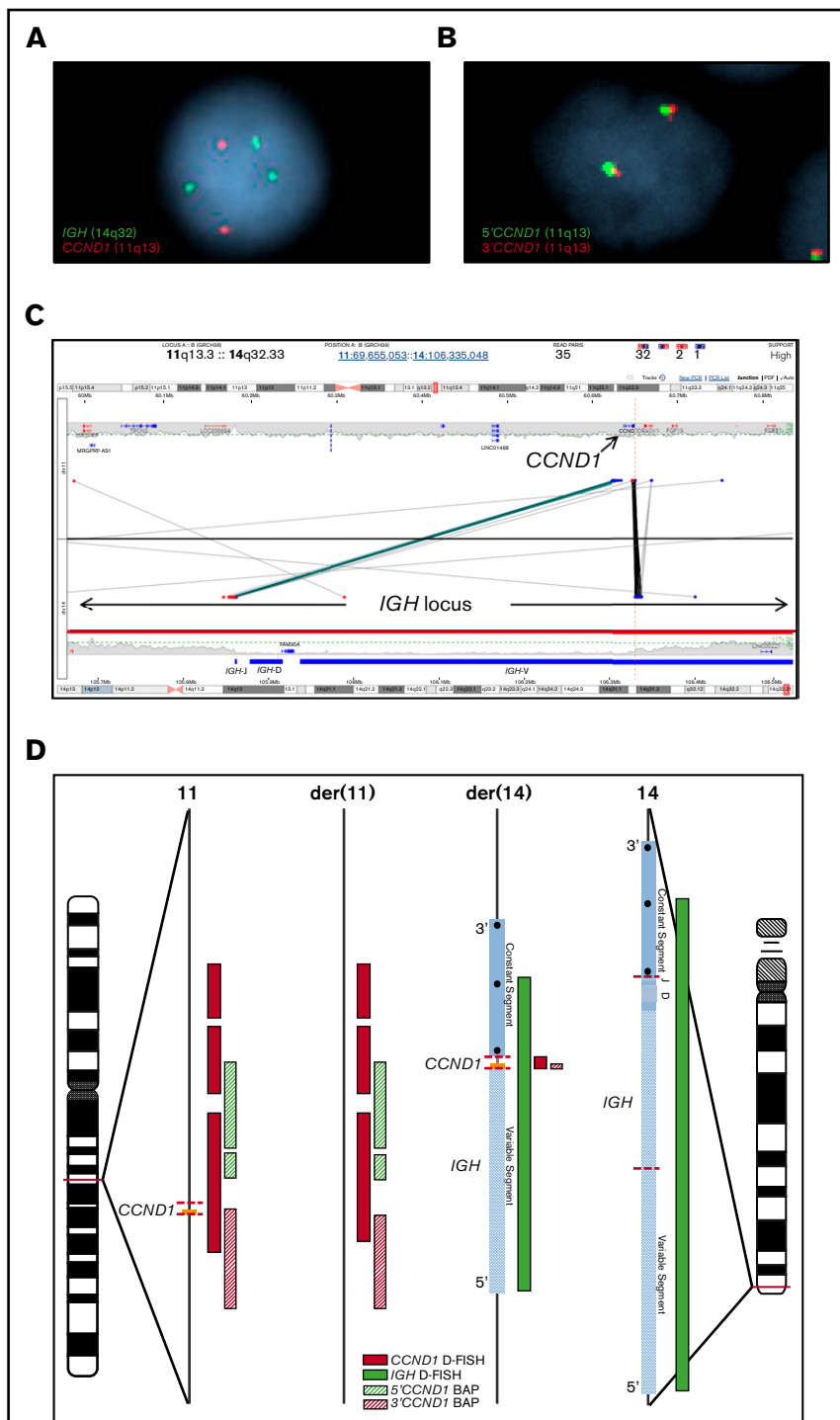


Figure 2. Cytogenomic evaluation. (A) Representative interphase cell demonstrating 2 red signals (*CCND1* probe, ~378 kb) and 3 green signals (*IGH* probe, ~1.6 Mb) using an *IGH/CCND1* dual-color dual-fusion probe set. This signal pattern indicates a gain of 14q32 or an *IGH* rearrangement, without the presence of *IGH/CCND1* fusion. The presence of an unbalanced or balanced t(11;14) would be indicated by 1 or 2 red/green (yellow) fusion signals, respectively. (B) Representative interphase cell demonstrating 2 intact yellow fusion signals (5' *CCND1* probe, ~384 kb [green]; 3' *CCND1* probe, ~256 kb [red]) using a *CCND1* break-apart probe (BAP) set. The presence of a *CCND1* rearrangement would be indicated by separated or single green (5' *CCND1* probe) and red (3' *CCND1* probe) signals. (C) Junction plot demonstrating the insertion of an intact *CCND1* gene into the *IGH* locus. In addition, loss of a portion of distal *IGH-J*, *IGH-D*, and a portion of the proximal *IGH-V* region was detected (2N indicated by the dashed gray horizontal line below the *IGH* locus label). (D) A depiction of the cryptic *CCND1* insertional event. Dashed horizontal red lines on the normal chromosome 11 indicate breaks encompassing the *CCND1* gene and insertion into the *IGH* locus located on the der(14). The black dots on *IGH* locus indicate enhancer elements. The *IGH* segment between the horizontal dashed red lines on chromosome 14 was deleted. The *IGH/CCND1* D-FISH and *CCND1* BAP footprints are indicated by the solid green/red and striped green/red horizontal bars, respectively. Insertion of the *CCND1* gene into the *IGH* locus is unappreciable by FISH because of the minimal size of the inserted *CCND1* gene segment. The secondary 12p/der(14q) translocation (not shown) also accounts for the extra *IGH* signal observed by *IGH/CCND1* D-FISH studies.

an in-house-developed pipeline, Mayo NGS Workbench, for base calling and insertion/deletion (indel) detection. Results were confirmed by reviewing the sequencing files (.bam file format) in Alamut Visual (Interactive Biosoftware, Rouen, France). This study was conducted in accordance with the Declaration of Helsinki.

Results and discussion

The discordant findings of strong cyclin D1 positivity by immunohistochemistry vs the lack of *CCND1* rearrangement by FISH were

resolved by MPseq results, which detected an insertional rearrangement involving chromosomes 11 and 14. This resulted in an intact coding region of the *CCND1* gene (NM_053056) from chromosome 11 inserted into the *IGH* locus on chromosome 14 (Figure 2C). The insertion of *CCND1* into the *IGH* locus characterized by MPseq (Figure 2D) explains the cyclin D1 overexpression observed by immunohistochemistry (Figure 1F). The identification of cryptic *IGH/CCND1* fusion by MPseq was critical in establishing a conclusive diagnosis of MCL in this

patient vs the diagnosis of cyclin D1–positive B-cell lymphoma established at presentation. The cryptic nature of this particular genetic alteration eluded detection by standard FISH techniques because of the minimal size of the *CCND1* gene segment inserted into the *IGH* locus (Figure 2D).

A subsequent balanced translocation was identified between 12p11.1 (intergenic region) and the *CCND1* 3' untranslated region (UTR) located on the derivative chromosome 14, resulting in a disrupted *CCND1* 3' UTR (data not shown). Both rearrangements were confirmed by Sanger sequencing and involved the same *CCND1* allele (supplemental Table 3). A retrospective analysis of the diagnostic bone marrow specimen by MPseq detected only the *CCND1* insertion event, indicating that the 12p/der(14q) translocation occurred secondarily during clonal evolution. Of interest, the translocation of the *CCND1* 3' UTR region and 12p11.2 chromosomal region has been described in cyclin D1–positive MCL without *IGH/CCND1* fusion and may indicate a rare but recurrent mechanism for cyclin D1 overexpression.¹¹ Truncation of the *CCND1* 3' UTR creates transcripts that lack messenger RNA destabilization elements, thus extending the half-life of the modified messenger RNA transcript and increasing its oncogenic effect.^{8,9}

Targeted panel NGS studies also revealed single nucleotide pathogenic mutations involving *CCND1* and *TP53* (supplemental Table 4). The combination of cryptic *IGH/CCND1* fusion, acquisition of the *CCND1* 3' UTR translocation involving 12p in conjunction with biallelic *TP53* alterations (deletion and point mutation), and the reported adverse effect of acquired *CCND1* mutations in MCL also explain the negative SOX11 expression by the lymphoma cells and may have contributed to progression after an initial response to ibrutinib therapy in this case.^{10,16} The patient was started on venetoclax, which resulted in a rapid decline of her WBC count, resolution of splenomegaly, and clinical improvement of her symptoms. Unfortunately, the response to venetoclax was not durable, and the patient expired as a result of progression of MCL 2 months later.

In conclusion, we have characterized a novel *IGH/CCND1* rearrangement using NGS that was undetectable by FISH and provided definitive evidence for a diagnosis of MCL. The comprehensive genetic characterization of this case revealed additional key findings that seem important in disease progression and therapeutic resistance.

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Authorship

Contribution: J.F.P. drafted and edited the manuscript, generated figures, and helped analyze the data; L.B.B. participated in writing and editing the manuscript and helped analyze the data; R.P.K. collected and analyzed data and edited the manuscript; B.A.P. and S.A.S. analyzed and interpreted data, generated figures, and edited the manuscript; G.V., J.B.S., and P.T.G. reviewed and edited the manuscript; A.A.M., C.A.T., and S.A.P. collected clinical data and participated in writing and editing the manuscript; and D.C. and D.S.V. collected and analyzed data, generated figures, and participated in writing and editing the manuscript.

Conflict-of-interest disclosure: Algorithms described in this manuscript for mate-pair sequencing are licensed to WholeGenome LLC owned by G.V. S.A.P. reports research funding to his institution from Pharmacyclics, MorphoSys, Janssen, AstraZeneca, and Ascentage Pharma for clinical studies in which he is a principal investigator. S.A.P. has also participated in Advisory Board meetings of Pharmacyclics, AstraZeneca, Genentech, Gilead, and AbbVie (not personally compensated for his participation). The remaining authors declare no competing financial interests.

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